PYCNOCLAVELLA BELIZEANA, A NEW SPECIES OF ASCIDIAN FROM THE CARIBBEAN

Ivan Goodbody

ABSTRACT

Pycnoclavella belizeana new species is described from mangrove lagoons on the Barrier Reef in Belize, Central America. The species is characterised by its small size, simple branchial sac with only three rows of stigmata, and white or orange pigment in the thorax. The last and sometimes the first row of stigmata may be deflected along the dorsal mid line of the thorax. Deflection of the stigmata is probably due to reduction in the size of the branchial sac and a need to fold the posterior and anterior rows of stigmata during contraction. *P. belizeana* is probably more closely related to *P. stanleyi* from the California coast than it is to *P. minuta* from West Africa.

Species in the genus *Pycnoclavella* have until recently been included in the ascidian Family Clavelinidae. However Kott (1990) has shown that on the basis of larval characteristics, and possibly also the process of asexual replication, *Pycnoclavella* probably had a separate evolutionary origin to *Clavelina* and related genera. On this basis Kott (1990) has erected a new Family, Pycnoclavellidae, containing two genera, *Pycnoclavella* Garstang, 1891, and *Euclavella* Kott, 1990. *Euclavella* is a monotypic genus containing a single species, *E. claviformis* Kott, 1990 from Australia and New Zealand. *Pycnoclavella* is a fairly widely distributed genus with species recorded from the English Channel (Garstang, 1891; Berrill, 1950), West Africa (Millar, 1953), California (Berrill and Abbott, 1949), the Mediterranean (Brunetti, 1991), the Red Sea (Kott, 1957), the Indo-Pacific (Sluiter, 1904; Van Name, 1918; Millar, 1975; Monniot, 1988) and Australia (Kott, 1990). For further discussion of the family and its characteristics the reader is referred to Kott (1990).

Hitherto there are no records of *Pycnoclavella* in the western Atlantic although its presence is to be expected in view of the widespread distribution of the genus. The present paper describes a new species *Pycnoclavella belizeana*, from mangrove lagoons on the Barrier Reef of Belize, Central America. The functional characteristics of the deflection of the dorsal ends of the anterior and posterior rows of stigmata in several species of *Pycnoclavella* is also discussed.

Pycnoclavella belizeana, new species

External Appearance.—Colonies consist of groups of very small zooids (*vide infra*) partially embedded in the surface of the peat bank at the edge of mangrove lagoons. Although the zooids are apparently derived from an asexual budding process each zooid has separated from others and leads an independent existence. The anterior thoracic region of the zooid protrudes above the surface of the peat and associated algal mat and although very small (ca. 1.0 mm) is readily recognisable due to an accumulation of either white or orange pigment in the tissues. The bulbous posterior abdomen is usually embedded in the algal mat or peat surface. Colonies may contain from less than 10 to more than 40 individual zooids. All recorded colonies have been seen in shallow water, less than 2 m.

Zooid.—A fully extended zooid is between 6.0 and 7.0 mm; the thoracic region containing the branchial sac is 1.0 mm in length and the abdominal region containing stomach and gonads is about 2.0 mm. These two regions, thorax and abdomen, are joined by a long, narrow oesophageal stalk (Fig. 1A).

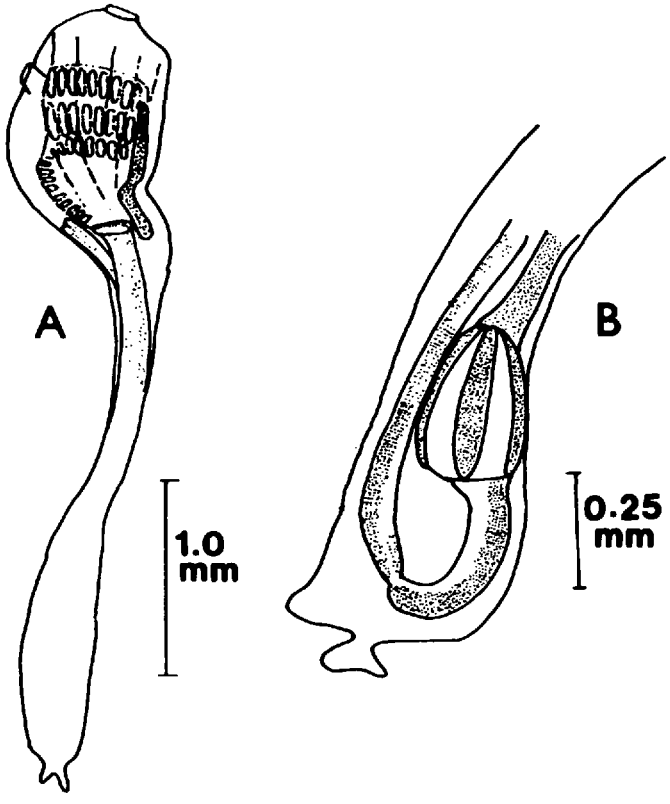

Figure 1. *Pycnoclavella belizeana* new species A. Whole zooid. B. Alimentary canal.

The branchial siphon is terminal at the anterior end, the atrial siphon is dorsal and slightly posterior to the branchial siphon; both siphons are smooth lipped, without any sign of lobation. There are 16 branchial tentacles of three different sizes. Two long tentacles are placed dorsally and ventrally respectively, so that their tips just meet in the center of the open siphon. Six medium length tentacles are arranged, three on either side, and the remaining eight short tentacles alternate with these and the long tentacles (Fig. 3B).

Branchial Sac.—The branchial sac has three rows of stigmata. In a typical specimen there are 18 stigmata in the first row, 13 in the second, and 24 stigmata in the posterior row, but the number is variable. In the fully expanded branchial sac these three rows of stigmata are arranged like a spindle so that the middle row is oriented in the anterior-posterior plane, while anterior and posterior rows are set at an angle of 45° or more. In the posterior row there are usually an indeterminate number of deflected stigmata whose orientation is such that they form an anterior-posterior sequence along the latero-dorsal margin of the branchial sac (Fig. 2). A similar torsion in the anterior row sometimes occurs so as to form a line of stigmata running forward from the first row. The significance of this arrangement of stigmata is returned to below in the Discussion. In between the rows of stigmata shelf-like membranes project radially into the branchial sac in a manner similar to that found in many species of *Clavelina.* The prepharyngeal groove is formed of a double membrane and the dorsal tubercle is a simple opening on the tip of a tubular prominence (Fig. 3A). Seen from the inside the dorsal side of the bran-

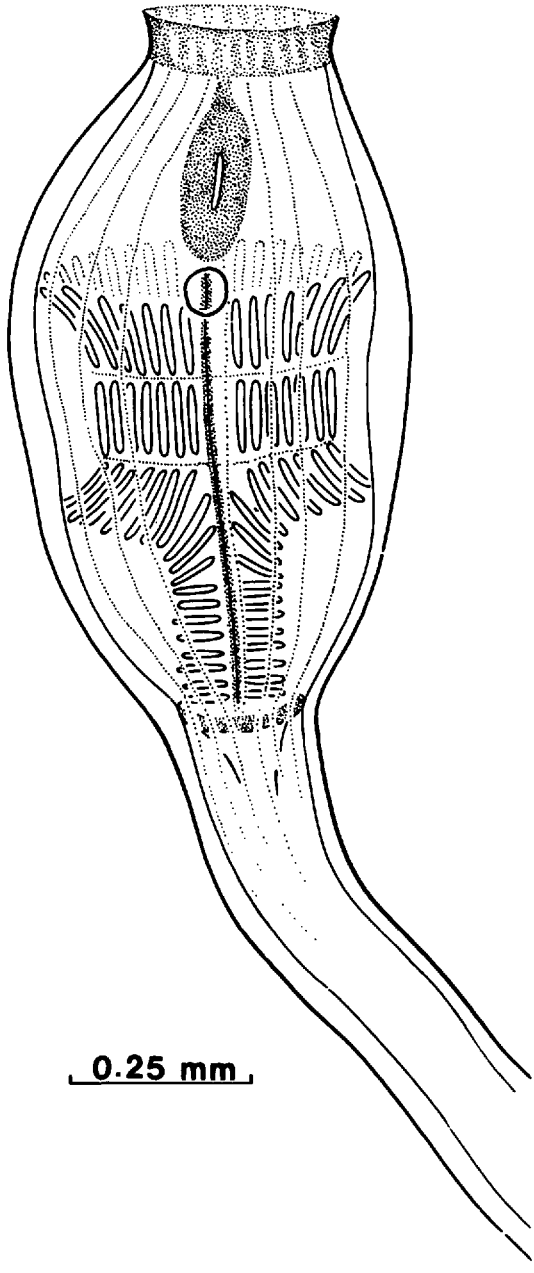

Figure 2. *Pycnoclavella belizeana* new species Dorsal view of the thorax showing the spindle-like arrangement of the three rows of stigmata.

chial sac has a groove bordered on either side by a membranous fold. The groove is bridged in four places by supports for tentacle-like languets (Fig. 3A). There are two large languets, one between each of the rows of stigmata; the other two languets are smaller, one near the anterior of the first row of stigmata, the other near the posterior of the last row. In the fully expanded zooid these smaller

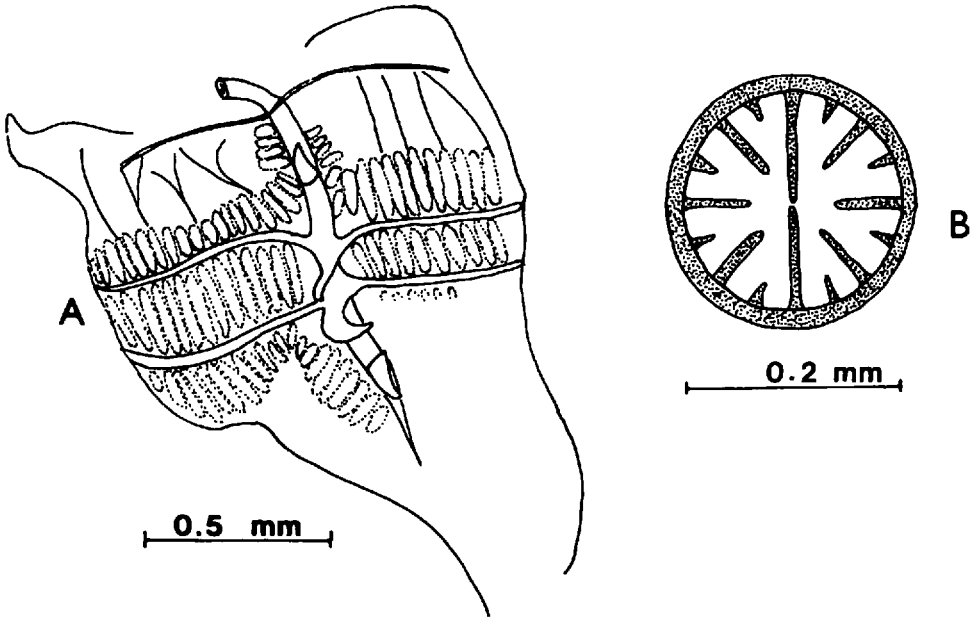

Figure 3. *Pycnoclavella belizeana* new species A. Branchial sac dissected from the ventral side to show the four languets along the dorsal groove. B. The arrangement of tentacles inside the branchial siphon.

languets are probably positioned at the anterior and posterior ends of the branchial sac respectively.

Mantle Muscle.—There are usually six to eight longitudinal fibres on each side of the thorax. These continue into the oesophageal stalk where there are numerous longitudinal fibers.

Alimentary Canal.—The oesophageal funnel is large and occupies most of the posterior end of the branchial sac; it leads into a long oesophagus. The stomach is box-like, each side formed of a bract shaped element giving the appearance of four ridges. A thin membranous post-stomach tapers to a narrow constriction at its junction with the intestine (Fig. 1B). The anus is bilobed and opens at the level of the posterior end of the branchial sac (Fig. 1A).

Gonads.—The gonads are situated on the left side of the gut loop (Fig. 4A). The testis forms a rosette of up to 25 follicles. The ovary has only a few eggs, six being the largest number recorded. Eggs are fertilized in the base of the oviduct where they commence development; development continues as the embryos pass up the duct.

Larva.—Sexual reproduction is either highly seasonal or is uncommon. In several hundred zooids collected in the months of April and May, I have seen no embryos. Several embryos have been recorded from specimens collected in February, but only one larva, incompletely developed, has been found. This larva (Fig. 4B) measures 580 μm, has a fully formed tail which wraps two-thirds around the trunk, has both ocellus and otolith but no other fully formed trunk structures. In particular, the adhesive structures, which are so characteristic of the genus, are not formed. A line of cells at the anterior end may represent the beginning of the

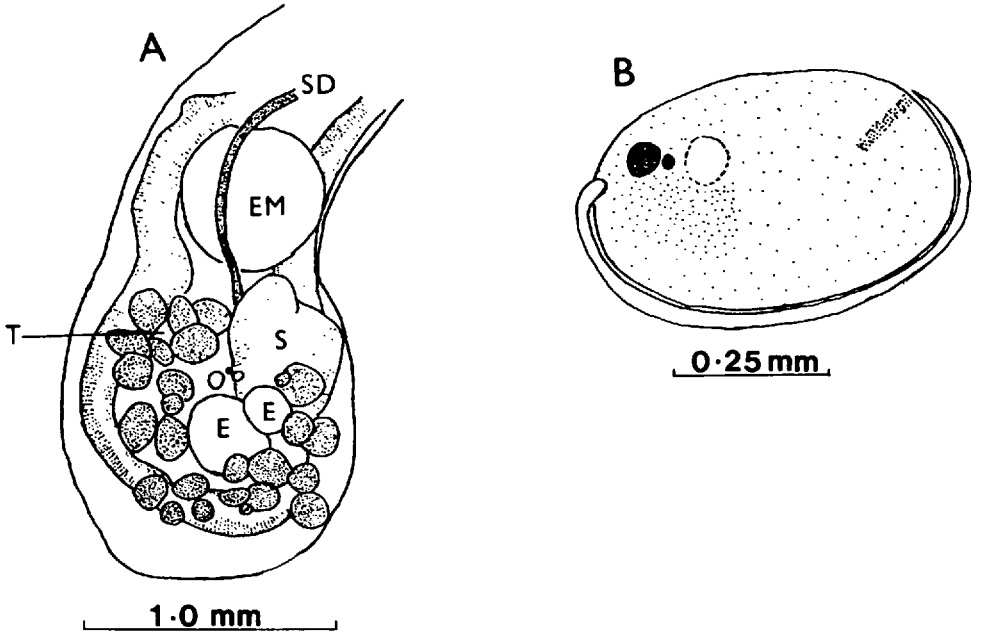

Figure 4. *Pycnoclavella belizeana* new species A. The arrangement of gonads in the gut loop. B. Developing larva. E: Eggs passing from ovary to oviduct [The ovary is the small group of cells above the two eggs]; EM: Embryo; S: Stomach; SD: Sperm duct; T: Testis lobes.

formation of one of the adhesive structures. However, it should be noted that, although these structures are not formed, the shape of the trunk conforms to that of *Pycnoclavella* and not to that of *Clavelina* (see Discussion below).

Habitat.—All recorded colonies have been on the margin of the peat bank at the edge of mangrove lagoons at Twin Cays and in the Pelican Cays on the Belize Barrier Reef. At Twin Cays most colonies are at the north end of the main channel where water depth is about 2 m and slow currents of water keep sedimentation to a minimum. Colonies are mostly in the upper meter of the vertical peat bank and are embedded in the superficial algal mat. This is a well shaded environment due to overhanging mangrove branches and roots. All of the zooids here are transparent with white pigment around the neural complex and endostyle only. At the Pelican Cays colonies have been recorded from an exposed eroded peat bank at the northern end of Fisherman's Cay. All colonies at this site are more opaque and the thorax is generally diffused with bright orange pigment. The difference in pigmentation at this site is probably a reflection of greater exposure to light.

Type Material.—The Type Series consists of a Holotype and eight Paratypes collected from the North Channel in Twin Cays, Belize, Central America. All were collected from the peat bank at the edge of the channel at a depth of approximately 0.5 m. Specimens were relaxed in menthol and fixed in formaldehyde and have been transferred to alcohol for permanent storage. They are deposited in the National Museum of Natural History, Washington D.C. and are designated as follows:

HOLOTYPE. Two zooids collected 23 April 1993. Both zooids are sexually im-

mature but are selected as the Holotype because they are well relaxed and demonstrate the structure of the branchial sac exceptionally well. [USNM 20599]

PARATYPES. (i) A single sexually mature zooid collected 23 April 1993 [USNM 20600]. (ii) A single zooid removed from the test to show the location of embryos; one late embryo removed to a separate vial. Collected 23 February 1988 [USNM 20601]. (iii) A sexually immature zooid showing the structure of the alimentary canal; collected 23 April 1993 [USNM 20602]. (iv) A single zooid containing embryos, removed from the test; collected 28 February 1988 [USNM 20603]. (v) A sexually mature zooid removed from the test; collected 28 February 1988 [USNM 20604]. (vi) A single zooid removed from the test to show the branchial sac; collected 28 February 1988 [USNM 20605]. (vii) A single zooid with intact test; collected 28 February 1988 [USNM 20606]. (viii) Five zooids showing posterior vascular stolons and other structures; collected 23 April 1993 [USNM 20607].

DISCUSSION

The family Pycnoclavellidae is defined by Kott (1990) as having small thread-like zooids divided into thorax and abdomen, with a long oesophageal neck, smooth apertures, no internal longitudinal branchial vessels, smooth-walled stomach, anus opening at the base of the branchial sac and fertilization taking place at the base of the oviduct. Many of these features are also found in the Clavelinidae but Pycnoclavellidae are distinguished by the larval adhesive organs, which are tubular and invaginated into the larval trunk. In Clavelinidae the adhesive organs are supported on a stalked frontal plate (Kott, 1990). In Clavelinidae also, fertilization occurs near the tip of the oviduct and development takes place in the atrial cavity or in an enlargement of the anterior end of the oviduct.

The new species from Belize cannot be a clavelinid because fertilization occurs at the base of the oviduct and the larva lacks the characteristic stalked frontal plate. On the other hand all the features of the new species conform to the Pycnoclavellidae, especially the three rows of stigmata with deflection of some stigmata at the dorsal end of the posterior row, anus opening at the base of the branchial sac and fertilization at the base of the oviduct. As noted above the only larva so far found is incompletely developed and adhesive structures are not formed. However if this animal was a clavelinid at this stage of larval development the stalked frontal shield would already be a conspicuous feature. The features of the larva, so far as it is developed, conform to those of pycnoclavellidae.

Kott (1990) divides the genus *Pycnoclavella* into three groups, based on colony form. The *Aurilucens* group have a common bassal test mass in which the zooids are embedded. The *Stanleyi* group has basal stolons but no common test mass. The *Detorta* group has the thorax turned through 90°. On this basis the new species appears to belong to the *Stanleyi* group and is more closely related to *P. stanleyi* (Berrill and Abbott, 1949) than to any other member of the genus.

Berrill and Abbott (1949) suggested that zooids of *P. stanleyi* had individually free thoraces but that the abdominal region was embedded in a common test. However Trason (1963) later showed that this was incorrect and that each zooid is completely free down to the posterior end of the abdomen where it is attached to a common basal network of test. Because *P. stanleyi* lives in sandy environments the interstices between zooids get filled with sand grains giving the impression that zooids are bound together by common test. In *P. belizeana* zooids are similarly free and while the thoraces extend freely above the peat substratum the interstices between the abdominal region become filled with algal and other

growths on the peat surface. *P. stanleyi* and *P. belizeana* are also similar in having bright pigment accumulated around the neural complex and along the endostyle. In *P. stanleyi* this pigment appears always to be orange (Berril and Abbott, 1949; Trason, 1963) but in *P. belizeana* it is white in colonies in Twin Cays and orange in colonies from the Pelican Cays. The accumulation of pigment around the neural complex is a common feature of tropical ascidians and is probably protective against strong illumination (c.Goodbody and Cole, 1987).

Zooids of *P. belizeana* consistently have only three rows of stigmata, and become sexually mature in that condition. In *P. stanleyi* the zooid and the early blastozooid have only three rows of stigmata but become more complex with growth resulting in partial division of some rows to give as many as seven rows along the dorsal side of the thorax but only three along the ventral side (Trason, 1963). This sort of complexity has never been seen in *P. belizeana.* The arrangement of branchial tentacles and of the dorsal tubercle is the same in both species.

Because the single larva of *P. belizeana* is not fully grown it is not possible to compare it with that of *P. stanleyi* described by Trason (1963). However it is significant that in *P. stanleyi* there is no otolith, only an ocellus, while *P. belizeana* has both otolith and ocellus.

One of the features of the genus *Pyconoclavella* is the deflection of the dorsal end of the posterior row of stigmata along the dorsal mid-line; a similar deflection is sometimes observed in the anterior row as well (Kott, 1990). The deflection of stigmata in very small zooids was first reported by Julin (1904) in *Archiascidia neapolitana*[1], a little known species with only two rows of stigmata. The condition is also reported in several other genera including *Eudistoma* and *Cystodytes* (Kott, 1990) and *Pseudodistoma* (Monniot, 1987). Brien (1948) considered that the existence of only two rows of stigmata in *Archiascidia* is neotenous but that the deflection of each row of stigmata, one anterior the other posterior, is a specialisation due to the growth in length of each row. Kott (1990) in discussing the same condition in *Pycnoclavella* considers it to be due to evolutionary reduction in the size of the branchial sac. The two views are similar in acknowledging that there is insufficient space in the branchial sac to accommodate the number of stigmata in a row. However observation of *Pycnoclavella belizeana* suggests that the condition is no more than a functional displacement of stigmata during partial contraction of the branchial sac. Examination of living zooids shows that the number of deflected stigmata is related to the state of contraction of the sac and that as the sac expands more and more of these deflected stigmata are brought back into the normal position. It is made possible by the fact that in the fully expanded branchial sac the first and last rows of stigmata stand almost at right angles to the anterior-posterior axis of the zooid. The row of stigmata on one side of the animal forms a semi-circle, the two halves a flat circle around the diameter of the zooid. Contraction of such a structure is impossible unless it can fold and each side folds independently at the dorsal end. This fold takes place in three dimensional space forming a curve and thus reducing to a minimum any distortion of the stigmata in the folded area (Fig. 2; Julin, 1904: fig. 1, for the same condition in *Archiascidia neapolitana*).

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[1] *Archiascidia* may be a synonym for *Pycnoclavella* (cf. Kott, 1990).

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Address: *Zoology Department, University of the West Indies, Kingston 7, Jamaica.*